



siRNA Product Instructions

Instructions

1. Product Description

The chemically synthesized siRNA is a ready-to-use double-stranded RNA of 21-25 bp, which is purified, annealed and configured into a liquid of working concentration, and can be directly used for transfection of cells. The product is lyophilized and stable. The dosage of the product is strictly measured and labeled with the number of moles. This product does not guarantee the knockdown of target gene protein level. (oligo synthesis parameters: rA molecular weight: 329.21, rG molecular weight: 345.21, rC molecular weight: 305.18, rU molecular weight: 306.17)

siRNA(nmol)	2.5	5	10	50	Final concentration
Amount of water added (μL)	125	250	500	2500	20 μM
	50	100	200	1000	50 μM
	25	50	100	500	100 μM

The calculation formula of resuspended siRNA buffer is: $\text{siRNA(nmol)}/\text{volume (L)} = 10^3\mu\text{M}(\mu\text{mol/L})$

Conversion of double-stranded siRNA units with approximately 21 bases: 1 OD \approx 2.5 nmol \approx 33 μg

2. Attention

- (1) The product is transported at room temperature in the form of freeze-dried powder. It is recommended to store in an environment below -20 °C. Pay attention to avoid repeated freezing and thawing to accelerate RNA degradation. Please pack and save. Centrifuge instantly before use, and use RNase-free Water or sterilized ddH₂O prepared into 20-100 μM storage liquid. The average molecular weight of siRNA is 13300. This product is provided in the form of dry powder, which is attached to the pipe wall and easily lost when opened. Therefore, please centrifuge before opening the lid, then carefully open the lid, and cover the lid for oscillation after dissolution;
- (2) We should strictly follow the rules of RNA operation and use the experimental consumables of RNase-free to carry out RNAi experiments to avoid RNA degradation. It is better to place the product on ice when using it;
- (3) RNA products with fluorescent labels (e.g., FAM, CY3, CY5) are packaged in brown microtubes by our company, as the fluorophores are light-sensitive and must be protected from light.

3. Controls

Negative control	The negative control is a siRNA with no homology to the target cells, used to exclude non-siRNA interference effects that may affect the expression level of the target gene in the experiment. When calculating the interference efficiency, the numerical calculation is defined as 1. In a rigorous RNAi experiment, negative control is essential.
Positive control GAPDH siRNA	The default is siRNA with interference efficiency of 90% of GAPDH, which is used to test the reliability of each operation step in the experimental process. When detecting the relative expression level of GAPDH by qPCR, other genes should be selected as reference genes.

FAM-negative control	Negative control with fluorescent modification: When detecting transfection efficiency, set up this group in parallel (note that it is not co-transfected with other groups), and operate in the dark. Observe under a fluorescence microscope or detect the transfection efficiency of siRNA by flow cytometry after 6-12 hours. The transfection efficiency of other experimental groups set up in parallel should theoretically be consistent with this group.
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4. Cell Experiments

In the transfection experiment, at least three replicate wells are set up for each transfected sample; when inoculating cells, the number of cells inoculated in each well is kept as consistent as possible, and the cells are distributed evenly on the surface of each well as much as possible. This reduces well-to-well variation and ensures experimental reliability and reproducibility. (Refer to the operation of Lipo2000 transfection reagent as follows, and refer to the instructions of transfection reagent for other transfection reagents).

1) Determination of transfection concentration

- (1) Please perform a gradient experiment to obtain the optimal amount of siRNA for transfection of cell lines. For example, use several concentrations of Lipo2000, or vary the siRNA concentration in the range of 20-100 nM to determine the conditions required to achieve optimal gene blocking levels. High concentrations of siRNA may be cytotoxic;
- (2) Fluorescently labeled siRNA as an indicator of transfection efficiency may be used to optimize transfection conditions and correct experimental problems in time.

2) Instructions for transfected cells

Adherent cells: 24 h before transfection, inoculate $0.5-2 \times 10^5$ cells in 400 μL of non-antibiotic medium with 60-80% cell fusion. When spreading the plate, the cells should be digested and mixed thoroughly to avoid accumulation of growth.

Suspension of cells: 24 h before transfection, inoculate $0.5-2 \times 10^5$ cells in 400 μL of antigen-free medium, and cell numbers to $4-8 \times 10^5$ /well for transfection.

3) Transfection steps

Take transfection reagent to transfect siRNA in 24-well plate, transfection concentration is 50 nM as an example, please refer to the following table for other container transfection specifications.

- (1) Dilute siRNA with 50 μL of Opti-MEM and gently blow 3-5 times to mix;
- (2) Invert the mixing of transfection reagent, dilute 1.0 μL of transfection reagent with 50 μL Opti-MEM, gently blow and aspirate 3-5 times to mix well, and let it stand for 5 min at room temperature;
- (3) Mix siRNA and transfection reagent, gently blow 3-5 times to mix well, and leave for 20 min at room temperature;
- (4) Add transfection complex in 24-well cell plate, 100 μL /well, mix thoroughly;
- (5) Cell plates were incubated in an incubator at 37°C, 5% CO_2 for 18-48 h. Fresh medium could be changed after 4-6 h of transfection.

	Total volume per well	Medium	Opti-MEM (diluted siRNA)	Opti-MEM (dilution of transfection reagents)	Final concentration	siRNA 20 μ M	Transfection reagent
96-well	100 μ L	50 μ L	25 μ L	25 μ L	100 nM	0.5 μ L	0.25 μ L
	100 μ L	50 μ L	25 μ L	25 μ L	50 nM	0.25 μ L	0.25 μ L
	100 μ L	50 μ L	25 μ L	25 μ L	30 nM	0.15 μ L	0.25 μ L
	100 μ L	50 μ L	25 μ L	25 μ L	20 nM	0.1 μ L	0.25 μ L
	100 μ L	50 μ L	25 μ L	25 μ L	10 nM	0.05 μ L	0.25 μ L
24-well	500 μ L	400 μ L	50 μ L	50 μ L	100 nM	2.5 μ L	1 μ L
	500 μ L	400 μ L	50 μ L	50 μ L	50 nM	1.25 μ L	1 μ L
	500 μ L	400 μ L	50 μ L	50 μ L	30 nM	0.75 μ L	1 μ L
	500 μ L	400 μ L	50 μ L	50 μ L	20 nM	0.5 μ L	1 μ L
	500 μ L	400 μ L	50 μ L	50 μ L	10 nM	0.25 μ L	1 μ L
12-well	1 mL	800 μ L	100 μ L	100 μ L	100 nM	5 μ L	2 μ L
	1 mL	800 μ L	100 μ L	100 μ L	50 nM	2.5 μ L	2 μ L
	1 mL	800 μ L	100 μ L	100 μ L	30 nM	1.5 μ L	2 μ L
	1 mL	800 μ L	100 μ L	100 μ L	20 nM	1.0 μ L	2 μ L
	1 mL	800 μ L	100 μ L	100 μ L	10 nM	0.5 μ L	2 μ L
6-well	2 mL	1500 μ L	250 μ L	250 μ L	100 nM	10 μ L	5 μ L
	2 mL	1500 μ L	250 μ L	250 μ L	50 nM	5 μ L	5 μ L
	2 mL	1500 μ L	250 μ L	250 μ L	30 nM	3 μ L	5 μ L
	2 mL	1500 μ L	250 μ L	250 μ L	20 nM	2 μ L	5 μ L
	2 mL	1500 μ L	250 μ L	250 μ L	10 nM	1 μ L	5 μ L

Note: The data in the table is for reference only, the amount of transfection reagents can be further optimized for some cell types.

5. Effectiveness test

Expression detection is performed 24–72 h after transfection, the optimal detection time is related to the cell type, transfection reagent and detection purpose.

- (1) Detection of RNA level: qPCR detection method was used, and a significant decrease in mRNA expression could be detected 24–72 h after siRNA transfection;
- (2) Detection of protein level: mainly Western Blot method, the detection time is affected by the amount of protein expression in the cell, half-life and other factors, generally 48–96 h;
- (3) Functional screening: EdU cell proliferation, EdUTP cell apoptosis and other methods were applied to screen cell function.

6. Animal experiment

It is recommended to use Chol, OME, PS and other modified siRNA for experiments to prolong the time limit.

Local administration: the most direct way of introduction, the introduction efficiency of siRNA is high, the dosage is small, and siRNA can be absorbed quickly. Suitable for superficial organs and tissues, including eyes, muscles and subcutaneous tissues.

Systematic drug delivery: some targets that cannot be reached by local drug delivery, such as viscera, organs and some hash distribution targets (such as lymphocytes, metastatic tumor cells, etc.), can be systematically injected and have a wide range of tissues, including heart, liver, spleen, lung and kidney.

7. Key points

(1) Ensure that the experiment is carried out in an RNase-free environment

There are a lot of RNase in the environment and air, and trace RNase can cause serious degradation of RNA. The RNA product must be diluted with DEPC water or RNase-free water. During the experiment, it is necessary to avoid the contact between RNA products and human skin or experimental consumables without RNase. Because the gas and droplets produced by breathing contain a lot of RNase, it is necessary to wear a mask in the ultra-clean workbench for experimental operation.

(2) Optimization of transfection conditions

It is extremely important to optimize transfection conditions. In different cells and experiments, it is suggested to optimize the optimal concentration of siRNA and transfection reagent. If the transfection efficiency of transfection reagent Poor, you can try to change other transfection reagents, different transfection reagents may have great differences in operation, and the experiment must be carried out in strict accordance with the instructions. If conditions permit, more efficient transfection methods can be replaced, such as electrotransmission or virus transfection.

(3) Phenotype validation using multiple siRNAs

The use of two or more siRNAs to validate a phenotype is recommended to rule out false positive results due to off-target effects. More and more journals now also tend to require the use of multiple siRNAs to validate the phenotype.

(4) Use of Antibody-Free Medium

Within 6-8 h of transfection, use an antifree medium. This is because antibiotics are toxic to cells and will affect the delivery of siRNA.

(5) Ensure healthy cell status

Use cells that have been passaged for 50 generations for experiments; the transfection efficiency of cells that have been passaged for too many generations will decrease. It is important to ensure that the cells are in a healthy state before transfection. Cells in poor state usually have low transfection efficiency and have a great impact on the detection of interference efficiency.

(6) Negative control, positive control and fluorescent control

The use of disordered siRNA as a negative control can eliminate the interference of non-targeting effects on the experimental data. The use of a positive control (our complimentary GAPDH siRNA) allows for quality control of the experimental process and helps to quickly analyze possible problems in the experiment. The use of fluorescent

control can analyze the transfection efficiency of siRNA, which is convenient for finding the best experimental conditions.

8. FAQ

1) Low cell transfection efficiency?

- (1) The purity of siRNA is too low, pay attention to avoid RNAase contamination when dissolving. Use DEPC-treated consumables and reagents.
- (2) Low concentration of siRNA transfection reagent complexes is required to optimize transfection efficiency and use the appropriate dose of transfection reagent.
- (3) Serum affects transfection efficiency and must be absent when preparing the siRNA-transfection reagent mixture. Serum can be present in the culture, but not antibiotics.
- (4) Improper storage of liposome transfection reagents, reagent failure, should be stored at 4 C.

2) Massive cell death after transfection?

- (1) Poor cell status, need to adjust the cell growth status: generally cells in good growth status have better tolerance to transfection reagents.
- (2) The concentration of siRNA or transfection reagent is too high.
- (3) Toxicity of transfection reagent is high, change the transfection reagent.
- (4) Low purity of siRNA, impurities affect the cell state.
- (5) Transfection time is too long, and fresh medium is not replaced in time after transfection.

3) After transfection, no fluorescence is observed?

- (1) Normally, FAM shows green fluorescence after transfection, and CY3 shows red fluorescence after transfection. When observing the fluorescence, first make sure the fluorescence excitation wavelength is correct, the green wavelength is 495 nm and the red wavelength is 550 nm.
- (2) Wash and observe the fluorescence within 12 hours after transfection to avoid prolonged incubation.
- (3) If the product is not stored properly, siRNA will be degraded and FAM will be quenched; FAM is easily decomposed by light, so please keep it away from light.
- (4) When transfection, avoid light operation, avoid FAM exposure to white light for a long time.
- (5) The transfection time is too long, and the fresh medium is not changed in time after transfection.

4) The interference effect of the target gene is only about 60%, is it judged that the package is invalid?

Usually, the package product guarantees that the interference effect can reach about 70%, if the interference effect reaches about 60%, we also think that the interference is meaningful. If there are obvious changes in the phenotype and function of the cells, it is also recognized in the literature. Generally, the interference efficiency can be improved by improving the transfection efficiency and optimizing the experimental system.

5) Why emphasize on mRNA level detection? Can we directly detect protein and function?

Since siRNA acts directly on mRNA, the mRNA level is the most direct indicator of validity. Many customers believe that the direct result of mRNA degradation should correspond to the decrease of protein level, so the result of

protein level can also be used as an indicator of the validity of the test. In fact, the mRNA drop does not correspond to the protein drop. Possible reasons for this are:

- (1) Relation to the time chosen for the assay. It may be that the decline in mRNA has not yet been reflected in a change in the amount of protein or has not reached a level sufficient for detection, so it is generally recommended that the time of protein and functional assays be pushed back slightly.
- (2) Relation to the expression of proteins in cells. The translation process of mRNA is very complex, and the expression of genes in the cell always needs to maintain a balance, and after the expression of certain proteins reaches a certain level, which is sufficient to maintain its cellular function, the expression function may be temporarily "turned off", and part of the transcribed mRNA may not participate in the protein translation process. Therefore, the down-regulation of mRNA levels is not completely positively correlated with the down-regulation of protein levels
- (3) More complex mechanisms may be involved.

6) How long can siRNA act in the cell? When is the best time to detect it?

RNAi mediated by siRNA is a transient phenomenon, which can not be stably passed, and generally its action time can not last for a long time. It is usually recommended to complete the detection within 3-4 d after transfection. The best detection time varies with cells and target genes, and it is usually detected between 24 and 24-48 h after transfection. It is generally recommended to detect mRNA level in 24-48 h and protein level in 48 ~ 72 h.

7) Is the transfection efficiency of cells related to siRNA sequence?

The transfection efficiency depends on the cell itself and transfection method, and is not directly related to the sequence of siRNA. Therefore, the transfection efficiency of siRNA in different cells may be different.

8) Why is the same siRNA effective in cell A but not in cell B?

Different cells have different transfection efficiency and different gene expression levels, which are related to the efficiency of siRNA, so the interference effect of different cells cannot be guaranteed to be consistent.

9. After-sales

After-Sales Terms: Given the nature of the product/service, if no quality issues are raised within one month of receiving the product/service deliverables, it will be deemed accepted without quality defects. For any quality issue jointly confirmed by both parties, we will either waive the fee for the defective product/service or re-provide the corresponding product/service once. We shall not be held liable for any additional responsibilities beyond these remedies.

Important Notice: This product/service is intended for research use only. It must not be used for clinical testing, diagnosis, or any other healthcare-related activities.

Contact information:

Contact E-mail: market@tsingke.com.cn; info@tsingke.com.cn

Website: www.tsingke.com

Address: Building 3, Yard No.105, Jinghai 3rd Road, Beijing E-Town, Beijing 100176 China